PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

B3

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 31/55

A1

(11) International Publication Number: WO 00/16781

(43) International Publication Date: 30 March 2000 (30.03.00)

(21) International Application Number: PCT/US99/21433

(22) International Filing Date: 17 September 1999 (17.09.99)

(30) Priority Data:
60/101,016 18 September 1998 (18.09.98) US

(71) Applicant *(for all designated States except US)*: SMITHKLINE BEECHAM CORPORATION [US/US]; One Franklin Plaza, Philadelphia, PA 19103 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: GILMARTIN, Aidan, G. [US/US]; 4F, 905 Clinton Street, Philadelphia, PA 19107 (US). HO, Maureen, L. [US/US]; 218 Shelly Drive, Collegeville, PA 19426 (US). IMBURGIA, Christina, S. [US/US]; 143 Abbey Drive, Royersford, PA 19468 (US). ROSHAK, Amy, K. [US/US]; 3004 Green Ridge Drive, Norristown, PA 19403 (US). LAGO, Maria, Amparo [ES/US]; 701 Pondview Drive, Audubon, PA 19403 (US).

(74) Agents: SIMON, Soma, G. et al.; SmithKline Beecham Corporation, Corporate Intellectual Property, UW2220, 709 Swedeland Road, P.O. Box 1539, King of Prussia, PA 19406-0939 (US).

(81) Designated States: CA, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: CHK1 KINASE INHIBITORS

(57) Abstract

Methods of using chk1 kinase receptor antagonists are provided.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

CHK1 KINASE INHIBITORS

FIELD OF THE INVENTION

The present invention relates to checkpoint kinase ("chk1 kinase") inhibitors, pharmaceutical compositions comprising these compounds and methods of using these compounds to treat various forms of cancer and hyperproliferative disorders.

BACKGROUND OF THE INVENTION

Eukaryotic cell cycle progression is controlled by a series of cell cycle-associated checkpoints which monitor and ensure the proper order and timing of events including the complete duplication and fidelity of DNA. Entry into mitosis is initiated by the M phase-promoting factor (MPF), a complex containing the cdc2 protein kinase and cyclin B. Proper regulation of MPF ensures that mitosis occurs only after earlier phases of the cell cycle are complete. Phosphorylation of cdc2 at Tyr-15 and Thr-14 suppresses this activity during interphase (G1, S, and G2). At G2-M transition, cdc2 is dephosphorylated at Tyr-15 and Thr-14 allowing MPF to phosphorylate its mitotic substrates. This is normally carried out by the dual specificity phosphatase cdc25C. However, in response to DNA damage the cell cycle arrests at the G2 phase and the inhibitory phosphorylation of the cdc2 kinase remains intact.

Recently, Sanchez *et al.* (*Science*,1997. 277:1497-1501) described the cloning and characterization of human checkpoint kinase (Chk1), a homologue of the *Schizosaccharomyces pombe* checkpoint kinase, which is essential for the DNA damage checkpoint. Human Chk1 has been shown to bind to and phosphorylate cdc25C on Ser 216 which results in the binding of a 14-3-3 protein (a family of proteins known to associate with cell cycle and cell death regulators, oncogenes and signaling molecules) to cdc25 and inhibition of the cdc25 phosphatase activity (Sanchez *et al. Science* 1997. 277:1497-1501; Peng *et al. Science*. 277:1501-1505). This maintains the cdc2 kinase in an inactive state preventing entry into mitosis. Overexpression of a S216A cdc25C mutant, which is unable to be phosphorylated by Chk1, resulted in the disruption of a G2 checkpoint and the inability of DNA damaged cells to arrest and repair at the G2 phase. One can therefore hypothesize that co-administration of a Chk1 inhibitor with a DNA damage agent would synergistically augment tumor cell death .

Chk1 kinase is an important cell cycle regulator, particularly at the G2/M phase. Inhibitors would therefore be attractive for the treatment of cancer. Current cancer

therapies, including surgery, radiation, and chemotherapy, are often unsuccessful in curing the disease. The patient populations are large. For example, in colon cancer alone there are 160,000 new cases each year in the US, and 60,000 deaths. There are 600,000 new colon cancer cases each year world wide. The number for lung cancer are twice that of colon cancer. The largest deficiency of chemotherapies for major solid tumors is that most patients fail to respond. This is due to cell cycle regulation and subsequent repair of damage to DNA or mitotic apparatus, the targets for most effective chemotherapeutic agents. Chk1 kinase offers a point of intervention upstream from these mechanisms by which tumor cells develop resistance. Inhibition of Chk1 could be used in conjunction with conventional chemotherapies to overcome drug resistance.

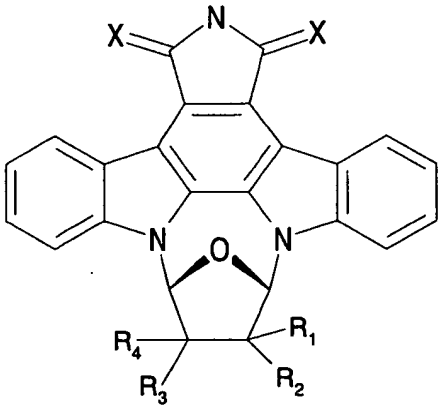
Based on the foregoing, there is a need to identify a potent chk1 kinase inhibitors for the treatment of the various aforementioned indications.

SUMMARY OF THE INVENTION

The present invention involves compounds represented by Formula (I) hereinbelow, pharmaceutical compositions comprising such compounds and methods of inhibiting chk1 kinase as well as specific assays to detect inhibition of chk1 kinase activity.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides compounds of Formula (I), hereinbelow:



Formula (I)

wherein:

X represents N, S or OH; and

R_1, R_2, R_3 and R_4 independently represent C_{1-6}alkyl, OH or SH or H.

Preferably, R_1, R_2, R_3 and R_4 independently represent C_{1-3}alkyl or H.

Most preferably, R_1, R_2, R_3 and R_4 represent H.

A particularly preferred compound useful in the present invention is: 9,10,11,12-Tetrahydro-9,12-epoxy-1H-diindolo[1,2,3-fg:3',2',1'-kl]pyrrolo[3,4-i][1,6]benzodiazocine-1,3(2H)-dione.

As used herein, "alkyl" refers to an optionally substituted hydrocarbon group joined together by single carbon-carbon bonds. The alkyl hydrocarbon group may be linear, branched or cyclic, saturated or unsaturated. Preferably, the group is saturated linear or cyclic.

The compounds of the present invention may contain one or more asymmetric carbon atoms and may exist in racemic and optically active forms. All of these compounds and diastereomers are contemplated to be within the scope of the present invention.

The present compounds can also be formulated as pharmaceutically acceptable salts and complexes thereof. Pharmaceutically acceptable salts are non-toxic salts in the amounts and concentrations at which they are administered.

Pharmaceutically acceptable salts include acid addition salts such as those containing sulfate, hydrochloride, fumarate, maleate, phosphate, sulfamate, acetate, citrate, lactate, tartrate, methanesulfonate, ethanesulfonate, benzenesulfonate, p-toluenesulfonate, cyclohexylsulfamate and quinate. Pharmaceutically acceptable salts can be obtained from acids such as hydrochloric acid, maleic acid, sulfuric acid, phosphoric acid, sulfamic acid, acetic acid, citric acid, lactic acid, tartaric acid, malonic acid, methanesulfonic acid, ethanesulfonic acid, benzenesulfonic acid, p-toluenesulfonic acid, cyclohexylsulfamic acid, fumaric acid, and quinic acid.

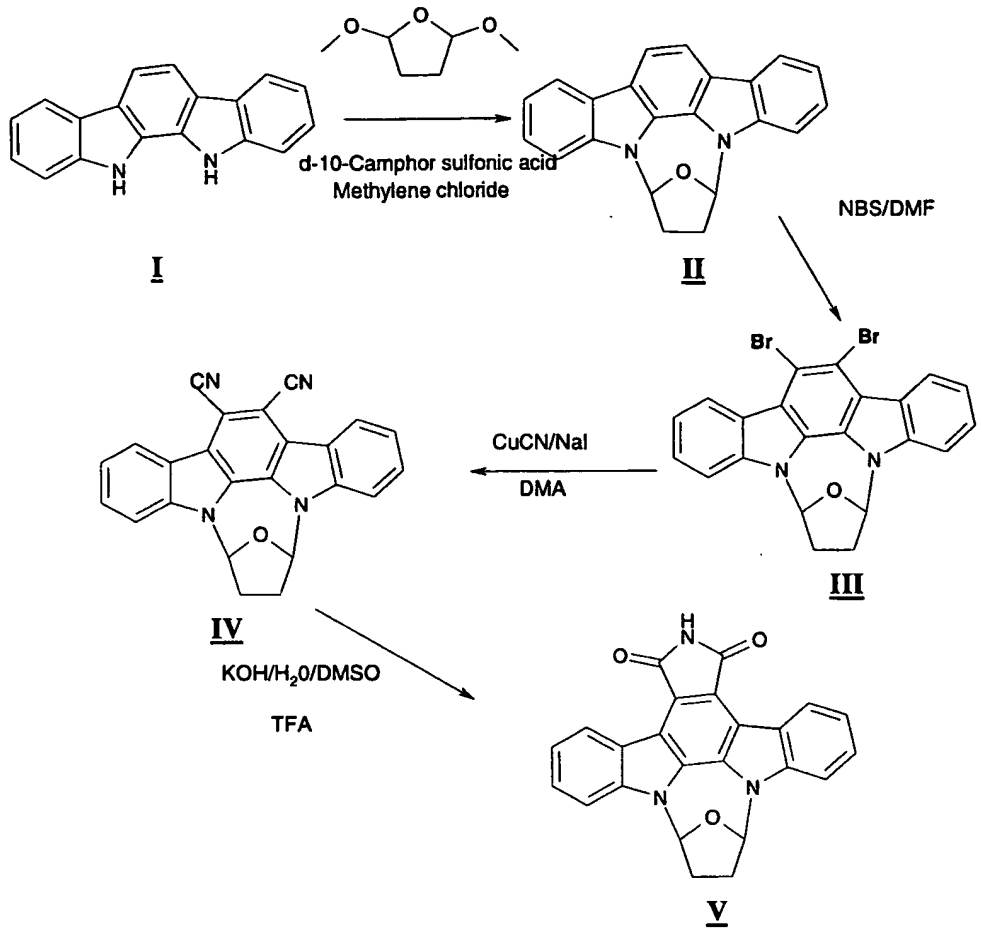
Pharmaceutically acceptable salts also include basic addition salts such as those containing benzathine, chloroprocaine, choline, diethanolamine, ethylenediamine, meglumine, procaine, aluminum, calcium, lithium, magnesium, potassium, sodium, ammonium, alkylamine, and zinc, when acidic functional groups, such as carboxylic acid or phenol are present.

The present compounds can be prepared by a process exemplified by Example I hereinbelow:

Example I

Synthesis of SB-218078 [9,10,11,12-Tetrahydro-9,12-epoxy-1H-diindolo[1,2,3-fg:3',2',1'-kl]pyrrolo[3,4-i][1,6]benzodiazocine-1,3(2H)-dione]

Reaction Scheme



A mixture of indolo[2,3-a] carbazol (SB-254273) (3.00g), 2,5-dimethoxytetrahydrofuran (2.41g) and d-10-camphor sulfonic acid (0.60g) in dry methylene chloride (200mL) was stirred at room temperature. After stirring overnight the mixture was evaporated and purified on a silica gel flash chromatography column using methylene chloride as eluent. The resulting colorless powder (II) (2.76g, m.p. 282-6°C) was used in next step.

To a stirred suspension of 11,12-(tetrahydrofuran-2,5-yl)-indolo[2,3-a]carbazole (II) (0.81g in 10mL DMF) was added, dropwise, a solution of N-bromosuccinimide (0.9g in 5mL DMF). The mixture was stirred at 65°C for 2hrs then cooled to room temperature and stirred overnight. Water (20mL) was added and after stirring for few minutes the mixture was filtered. The precipitate was washed with water and dried in vacuo resulting in 1.2g of compound III which was used in next step.

The mixture of compound III (1.2g), copper (I) cyanide (0.90g) and sodium iodide (0.75g) in dry dimethyl acetamide (DMA) was heated to 165°C under nitrogen. After 24 hrs the mixture was cooled to r.t and the precipitated yellow residue was washed with water and dried under vacuo (0.82g). The mass-spectrum of this product indicated mainly the desired product (IV, di-cyano compound, SB-254273) with some trace amount of mono-cyano analog.

The dicyano compound (IV, 0.38g) was heated to 150°C for 1.5 hrs in DMSO (18mL), water (1mL) and KOH (0.57g). After cooling to r.t., trifluroacetic acid (1mL) was added drop wise. The resulting solution was stirred at r.t. overnight. Water (40mL) was added and the precipitated yellow residue was collected, washed with water and dry in vacuo to afford compound V, 0.37g, m.p. > 300°C. The crude product was recrystallized from DMF/water (5mL 10:1) to afford a yellow crystalline material (0.10g), m.p. >300°C.

See also, McCombie, Stuart W.; Bishop, Robert W.; Carr, Donna; Dobek, Emily; Kirkup, Michael P.; Kirschmeier, Paul; Lin, Sue Ing; Petrin, Joanne; Rosinski, Karen; et al. Indolocarbazoles. 1. Total synthesis and protein kinase inhibiting characteristics of compounds related to K-252c. Bioorg. Med. Chem. Lett. (1993), 3(8), 1537-42, incorporated herein in its entirety by reference.

With appropriate manipulation and protection of any chemical functionality, synthesis of the remaining compounds of Formula (I) is accomplished by methods analogous to those above.

In order to use a compound of Formula (I) or a pharmaceutically acceptable salt thereof for the treatment of humans and other mammals, it is normally formulated in accordance with standard pharmaceutical practice as a pharmaceutical composition.

The present ligands can be administered by different routes including intravenous, intraperitoneal, subcutaneous, intramuscular, oral, topical, transdermal, or transmucosal administration. For systemic administration, oral administration is preferred. For oral administration, for example, the compounds can be formulated into conventional oral dosage forms such as capsules, tablets and liquid preparations such as syrups, elixirs and concentrated drops.

Alternatively, injection (parenteral administration) may be used, e.g., intramuscular, intravenous, intraperitoneal, and subcutaneous. For injection, the compounds of the invention are formulated in liquid solutions, preferably, in physiologically compatible buffers or solutions, such as saline solution, Hank's solution, or

Ringer's solution. In addition, the compounds may be formulated in solid form and redissolved or suspended immediately prior to use. Lyophilized forms can also be produced.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, bile salts and fusidic acid derivatives. In addition, detergents may be used to facilitate permeation. Transmucosal administration, for example, may be through nasal sprays, rectal suppositories, or vaginal suppositories.

For topical administration, the compounds of the invention can be formulated into ointments, salves, gels, or creams, as is generally known in the art.

The amounts of various compounds to be administered can be determined by standard procedures taking into account factors such as the compound IC_{50}, EC_{50}, the biological half-life of the compound, the age, size and weight of the patient, and the disease or disorder associated with the patient. The importance of these and other factors to be considered are known to those of ordinary skill in the art.

Amounts administered also depend on the routes of administration and the degree of oral bioavailability. For example, for compounds with low oral bioavailability, relatively higher doses will have to be administered.

Preferably the composition is in unit dosage form. For oral application, for example, a tablet, or capsule may be administered, for nasal application, a metered aerosol dose may be administered, for transdermal application, a topical formulation or patch may be administered and for transmucosal delivery, a buccal patch may be administered. In each case, dosing is such that the patient may administer a single dose.

Each dosage unit for oral administration contains suitably from 0.01 to 500 mg/Kg, and preferably from 0.1 to 50 mg/Kg, of a compound of Formula (I) or a pharmaceutically acceptable salt thereof, calculated as the free base. The daily dosage for parenteral, nasal, oral inhalation, transmucosal or transdermal routes contains suitably from 0.01 mg to 100 mg/Kg, of a compound of Formula(I). A topical formulation contains suitably 0.01 to 5.0% of a compound of Formula (I). The active ingredient may be administered from 1 to 6 times per day, preferably once, sufficient to exhibit the desired activity, as is readily apparent to one skilled in the art.

As used herein, "treatment" of a disease includes, but is not limited to prevention, retardation and prophylaxis of the disease. As used herein, "diseases" treatable using the present compounds include, but are not limited to leukemias, solid tumor cancers and metastases, lymphomas, soft tissue cancers, brain cancer, esophageal cancer, stomach cancer, pancreatic cancer, liver cancer, lung cancer, bladder cancer, bone cancer, prostate cancer, ovarian cancer, cervical cancer, uterine cancer, testicular cancer, kidney cancer, head cancer and neck cancer, chronic inflammatory proliferative diseases such as psoriasis and rheumatoid arthritis; proliferative cardiovascular diseases such as restenosis; prolifertive ocular disorders such as diabetic retinopathy; and benign hyperproliferative diseases such as hemangiomas.

Composition of Formula (I) and their pharmaceutically acceptable salts which are active when given orally can be formulated as syrups, tablets, capsules and lozenges. A syrup formulation will generally consist of a suspension or solution of the compound or salt in a liquid carrier for example, ethanol, peanut oil. olive oil, glycerine or water with a flavoring or coloring agent. Where the composition is in the form of a tablet, any pharmaceutical carrier routinely used for preparing solid formulations may be used. Examples of such carriers include magnesium stearate, terra alba, talc, gelatin, acacia, stearic acid, starch, lactose and sucrose. Where the composition is in the form of a capsule, any routine encapsulation is suitable, for example using the aforementioned carriers in a hard gelatin capsule shell. Where the composition is in the form of a soft gelatin shell capsule any pharmaceutical carrier routinely used for preparing dispersions or suspensions may be considered, for example aqueous gums, celluloses, silicates or oils, and are incorporated in a soft gelatin capsule shell.

Typical parenteral compositions consist of a solution or suspension of a compound or salt in a sterile aqueous or non-aqueous carrier optionally containing a parenterally acceptable oil, for example polyethylene glycol, polyvinylpyrrolidone, lecithin, arachis oil or sesame oil.

Typical compositions for inhalation are in the form of a solution, suspension or emulsion that may be administered as a dry powder or in the form of an aerosol using a conventional propellant such as dichlorodifluoromethane or trichlorofluoromethane.

A typical suppository formulation comprises a compound of Formula (I) or a pharmaceutically acceptable salt thereof which is active when administered in this way, with a binding and/or lubricating agent, for example polymeric glycols, gelatins, cocoa-butter or other low melting vegetable waxes or fats or their synthetic analogs.

Typical dermal and transdermal formulations comprise a conventional aqueous or non-aqueous vehicle, for example a cream, ointment, lotion or paste or are in the form of a medicated plaster, patch or membrane.

Preferably the composition is in unit dosage form, for example a tablet, capsule or metered aerosol dose, so that the patient may administer a single dose.

No unacceptable toxological effects are expected when compounds of the present invention are administered in accordance with the present invention.

The biological activity of the compounds of Formula (I) are demonstrated by the tests indicated hereinbelow.

Chk1 Methods:

Compounds capable of inhibiting myt1 kinase can be identified with in vitro assays and cellular assays as described below. Variations of these assays would be obvious to those skilled in the art.

Expression of GST-Chk1:

A GST-Chk1 expression construct was constructed which has the glutathione-S-transferase gene fused to the amino terminus of Chk1 kinase via a linker containing a thrombin cleavage site. This construct was cloned into the Baculovirus expression vector, pFASTBAC, and this was used to make the viral stock for the subsequent infection. Spodoptera frugiperda cells (Sf9) were infected with the virus expressing the GST-Chk1 and the cells were grown for 3 days, then harvested and frozen down.

Purification of GST-Chk1:

The GST-Chk1 protein was purified as follows: An Sf9 cell pellet expressing GST-Chk1 was resuspended on ice in lysis buffer (50mM Tris-Cl, pH 7.5, 250mM $NaCl_2$, 1mM dithiothreitol (DTT), 0.1%Brij, 5% (v/v) protease inhibitor cocktail, 1mM sodium orthovanadate), cells were lysed by sonication and centrifuged at 100,000xg for 30min The supernatant was added to Glutathione Sepharose 4B, beads, equilibrated in wash buffer (20mM Tris-Cl, pH 7.0, 10mM $MgCl_2$, 100mM $NaCl_2$, 1mM DTT, 0.5%(v/v) protease inhibitor cocktail, 1mM sodium orthovanadate). The mixture was rocked for 30min The resin with the bound GST-Chk1 was spun down at 500xg for 5min and washed with 14mls of wash buffer. The beads were spun as above and resuspended in another 14mls of wash buffer. The suspension was transferred into a column and allowed to pack, then the wash buffer was allowed to flow through by gravity. The GST-Chk1 was eluted from the column with 10mM Glutathione in 50mM Tris-Cl, pH 8.0 in 500ul fractions. Protein concentrations were determined on the fractions using Bio-Rad's Protein assay kit as per

instructions. Fractions containing the GST-Chk1 were pooled and diluted to a concentration of ~0.5mg/ml and dialyzed for 4 hours at 4^0C in dialysis buffer (20mM HEPES, pH 7.0, 1mM Manganese Acetate, 100mM $NaCl_2$, 0.05% Brij-35, 10% glycerol, 1mM DTT, 0.2% (v/v) protease inhibitor cocktail, 1mM sodium orthovanadate). The protein was aliquoted and stored at -80^0.

Cell Cycle Studies:

Drug studies considering cellular effects were performed in the Hela S3 adherent cell line. Cells were plated at a concentration sufficiently low such that 24 hours later they were at 10-20% confluence (typically $2x10^5$ cells/15cm e3). Cells were then synchronized in S phase by a repeated thymidine block. Briefly, cells were treated with 2mM thymidine for 18hours, released for 8 hours by 3 washes, and then treated again with thymidine. Following the second release from thymidine, 95% of cells were in S phase. Synchronized cells were then returned to complete media containing a DNA-damaging drug such as 50nM topotecan (a dosage we have found to be sufficient to arrest cells in early G2 phase without inducing apoptosis) alone and in combination with test compounds for up to 18 hours. Cell cycle profiles were then performed cytometrically using a procedure for propidium iodide staining of nuclei. (Vindelov et al, Cytometry Vol.3, No.5, 1983, 323-327) CHK1 inhibitors would be expected to reverse the G2 arrest caused by the DNA damaging agent. Typical concentration ranges for such activity would be 0.001 to 10 uM.

Proliferation/Apoptosis Studies:

Proliferation studies were performed in a variety of adherent and non-adherent cell lines including Hela S3, HT29, and Jurkat. The proliferation assay utilized a colorimetric change resulting from reduction of the tetrazolium reagent XTT into a formazan product by metabolically active cells

(Scudiero et al. Cancer Research, 48, 1981, 4827-4833) Cells were seeded in 100ul in 96 well plates to roughly 10% confluence (cell concentration varied with cell lines) and grown for 24 hours. Compounds were then added with or without sufficient vehicle- containing media to raise the cells to a 200ul final volume containing chemical reagents in 0.2% DMSO. Cells received multiple concentrations of DNA-damaging anti-proliferative drugs such as topotecan, test compounds, and combination treatment at 37°C 5% CO_2. 72 hours later, 50 uls of an XTT/ phenazine methosulfate mixture were added to each well and cells were left to incubate for 90mins. Plate was read at 450nm, and anti-proliferative effects were compared relative to vehicle treated cells. CHK1 inhibitors are expected to enhance

the cytotoxicity of DNA-damaging chemotherapeutic drugs. Typical concentration ranges for such activity would be 0.001 to 10 uM. Other assays for cellular proliferation or cytotoxicity could also be used with test compounds, and these assays are known to those skilled in the art.

Chk1 Kinase Assay:

Each well of a 96 well Flashplate (Amersham, Arlington Heights, VA) was coated with 1ug of the GST-cdc25C fusion protein diluted in PBS. Plates were incubated overnight at 4°C then washed twice in PBS and dried for 5-30 minutes at 37°C. DMSO vehicle or compounds were added as 2ul/well prior to addition of 0.1uCi/well of [^{33}P]-γATP and 10uM cold ATP and kinase reaction buffer containing 20mM HEPES (pH 7.4), 50 mM KCl, 10 mM $MgCl_2$, 1 mM EGTA, 0.5 mM DTT. The reaction was initiated by addition of GST-CHK1 (0.5 ug/well) and was allowed to proceed for a time predetermined to be linear on a time vs phosphorylation plot. Reaction is terminated with the addition of an equal volume (50 uL) of 50 mM EDTA. Plates were washed four times in PBS, dried for 30 minutes at 30°C and quantitated by liquid scintillation counting. Typical concentration ranges in which test compounds are expected to inhibit CHK1 activity are 0.001 to 10 uM.

Formulations for pharmaceutical use incorporating compounds of the present invention can be prepared in various forms and with numerous excipients. Examples of such formulations are given below:

Example 2

Inhalant Formulation:

A compound of Formula (I), (1 mg to 100 mg) is aerosolized from a metered dose inhaler to deliver the desired amount of drug per use.

Example 3

Tablet Formulation:

Tablets/Ingredients		Per Tablet
1.	Active ingredient (Cpd of Form. (I)	40 mg
2.	Corn Starch	20 mg
3.	Alginic acid	20 mg
4.	Sodium Alginate	20 mg
5.	Mg stearate	1.3 mg

Procedure for Tablet Formulation:

Ingredients 1, 2, 3 and 4 are blended in a suitable mixer/blender. Sufficient water is added portion-wise to the blend with careful mixing after each addition until the mass is of a consistency to permit its conversion to wet granules. The wet mass is converted to granules by passing it through an oscillating granulator using a No. 8 mesh (2.38 mm) screen. The wet granules are then dried in an oven at 140°F (60 °C) until dry. The dry granules are lubricated with ingredient No. 5, and the lubricated granules are compressed on a suitable tablet press.

Example 4

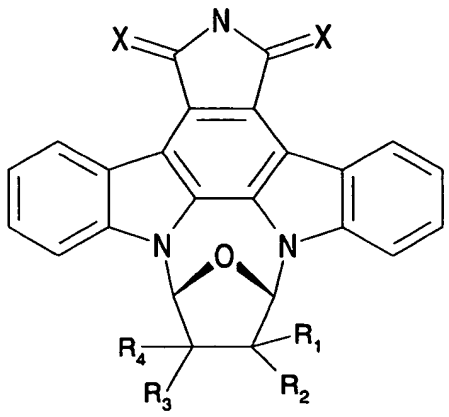
Parenteral Formulation

A pharmaceutical composition for parenteral administration is prepared by dissolving an appropriate amount of a compound of Formula I in polyethylene glycol with heating. This solution is then diluted with water for injections (to 100 mL). The solution is then rendered sterile by filtration through a 0.22 micron membrane filter and sealed in sterile containers.

All publications, including but not limited to patents and patent applications cited in this specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference as though fully set forth.

What is claimed is:

1. A method of inhibiting chk1 kinase activity which comprises administering to a subject in need thereof, an effective amount of a compound according to Formula (I) hereinbelow:



Formula (I)

wherein:

X represents N, S or OH; and

R_1, R_2, R_3 and R_4 independently represent C_{1-6}alkyl, OH or SH or H.

2. A method according to claim 1 wherein R_1, R_2, R_3 and R_4 independently represent C_{1-3}alkyl or H.

3. A method according to claim 2 wherein R_1, R_2, R_3 and R_4 represent H.

4. A method according to claim 3 wherein the compound is: 9,10,11,12-Tetrahydro-9,12-epoxy-1H-diindolo[1,2,3-fg:3',2',1'-kl]pyrrolo[3,4-i] [1,6]benzodiazocine-1,3(2H)-dione.

4. A method of treating a disease or disorder selected from the group consisting of leukemia, solid tumor cancer, metastases, lymphomas, soft tissue cancers, brain cancer, esophageal cancer, stomach cancer, pancreatic cancer, liver cancer, lung cancer, bladder cancer, bone cancer, prostate cancer, ovarian cancer, cervical cancer, uterine cancer, testicular cancer, kidney cancer, head cancer and neck cancer, chronic inflammatory proliferative diseases, proliferative cardiovascular diseases, proliferative ocular disorders and benign hyperproliferative diseases which comprises administering to a subject in need thereof an effective amount of a compound according to claim 1.

5. A method according to claim 4 wherein the disease or disorder treated is selected from the group consisting of psoriasis, rheumatoid arthritis, diabetic retinopathy and hemangiomas.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/21433

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 31/55
US CL : 514/211

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/211

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,621,101 A (LEWIS et al.) 15 April 1997, see the entire document.	1-5

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 03 DECEMBER 1999	Date of mailing of the international search report 03 FEB 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer ZOHREH FAY Telephone No. (703) 308-1235